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Biofilm formation in MRSA isolated in Cystic Fibrosis patients is strain-dependent and differentially influenced by antibiotics

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INTRODUCTION

- Staphylococcus aureus* is the most frequent pathogen in cystic fibrosis (CF) and multidrug resistant strains especially **methicillin-resistant S. aureus** (MRSA) have been associated with more severe lung dysfunction¹.
- MRSA eradication is thus recommended based on specific therapeutic strategies.
- Lung abnormalities in CF include a sticky mucus → microorganisms are protected from eradication treatments.
- Bacterial traits, including the **ability to form biofilm**, are also important features of persistence^{2,3}.

RESULTS

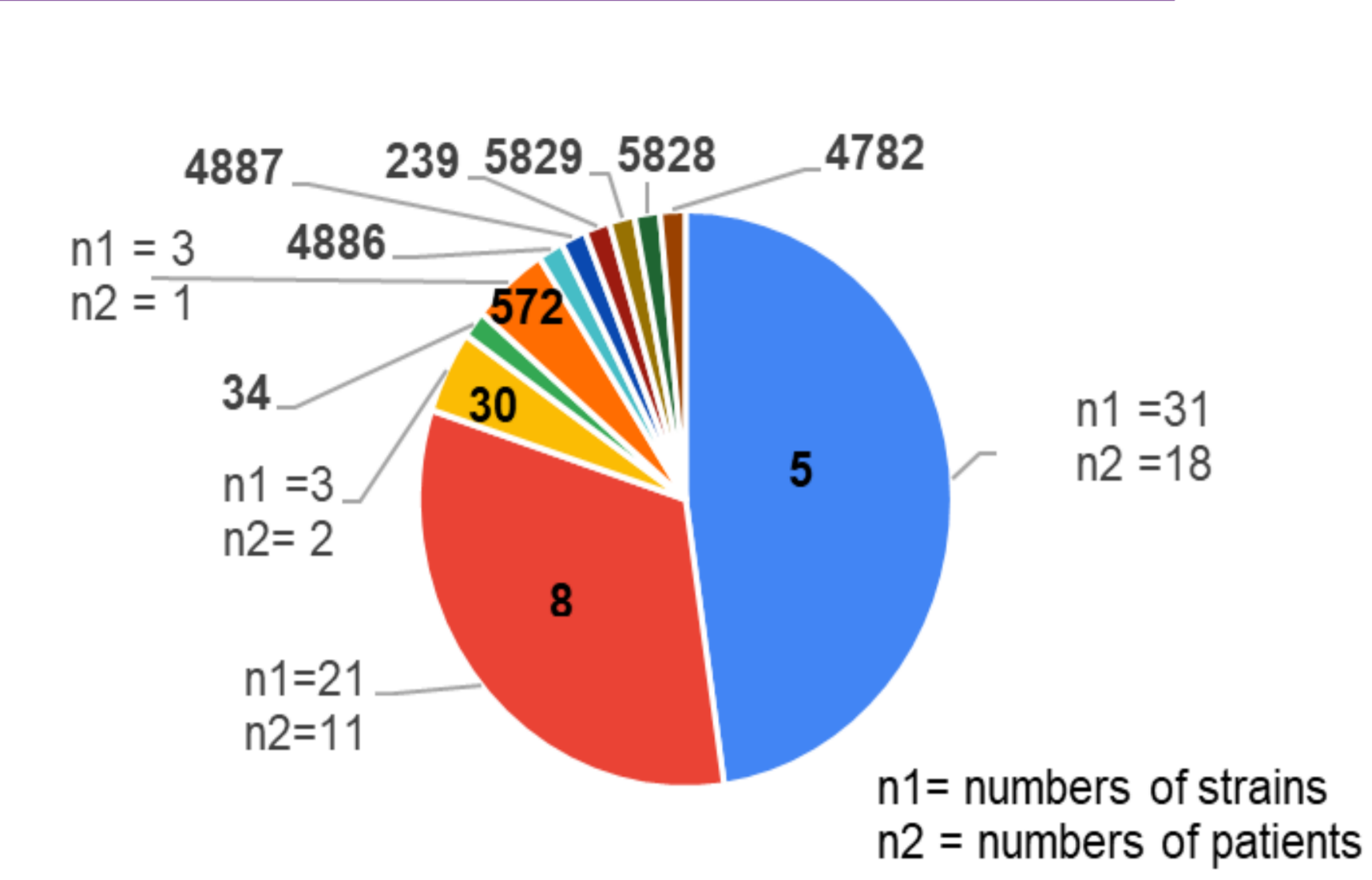


Figure 1: Sequence type (ST, bold type) distribution of 65 SARM isolated in 35 CF patients. n1= no of strains, n2= no of patients (indicated when >1 strain/ST)

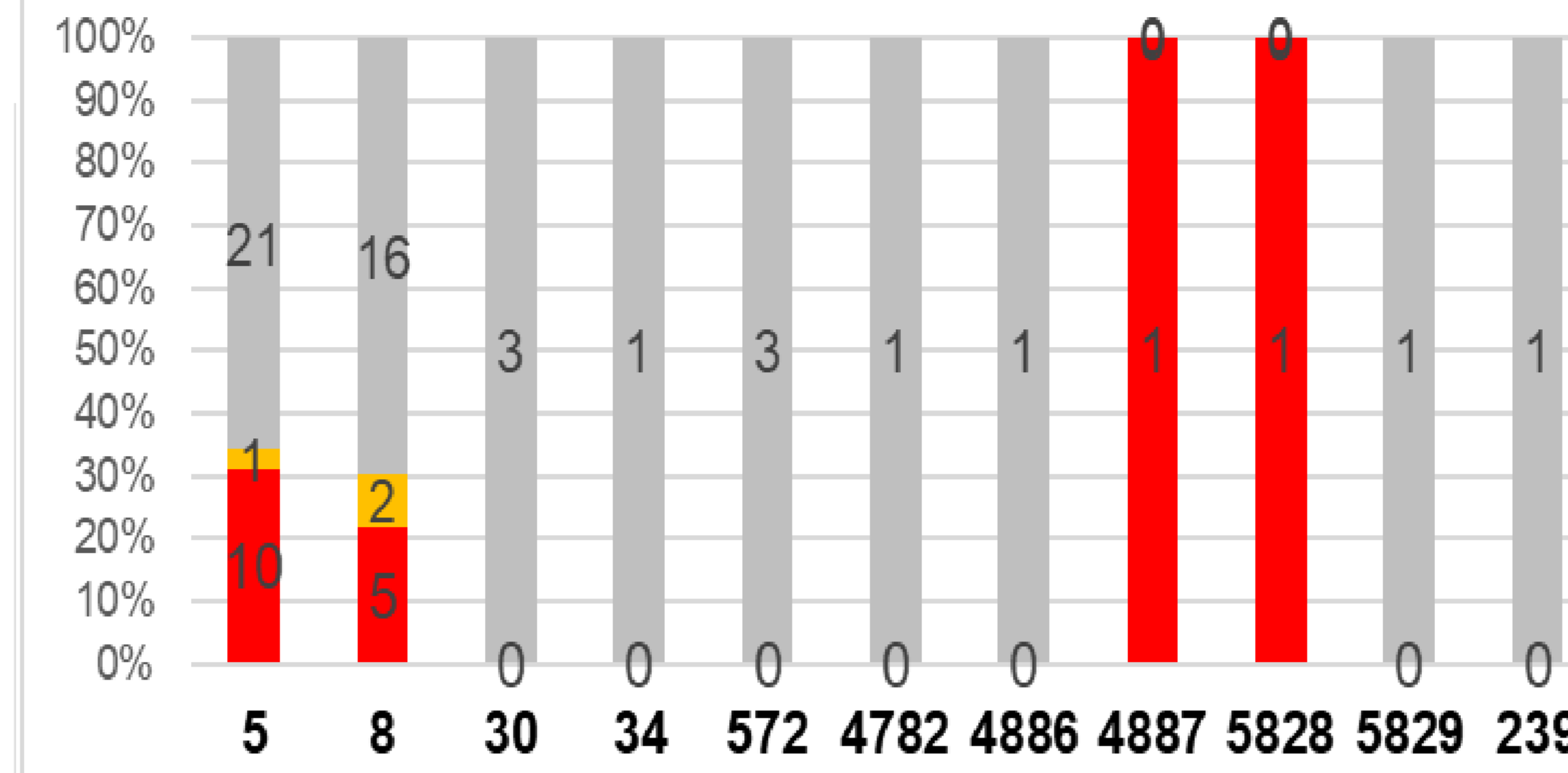


Figure 2: Relative distribution of MRSA strains according to their ST and ability to form biofilm using BRT®. Grey = no biofilm production (BP), orange = partial BP, red = complete BP.

- Strains belong to 11 STs (Figure 1). 3 patients had co-colonizations with strains of different STs within the same Clonal Complex (CC) → 5 & 4782, 5 & 4887, 8 & 5829.
- ST5 and ST8 were the most frequent STs in our study (Figure 1): 80% of the strains (52 strains/65) 83% of the patients (29 patients/35)
- Strains of ST5 & ST8 were more often producers of biofilm: 23.4% (15 strains/65) / 31.4% (11 patients/35) (Figure 2)
- 17/65 strains (26%) (13 patients (37%)) formed mature biofilm in 4h in ASM (Figure 3).
- 4 patients had strains displaying distinct ability to form biofilm within a sample.

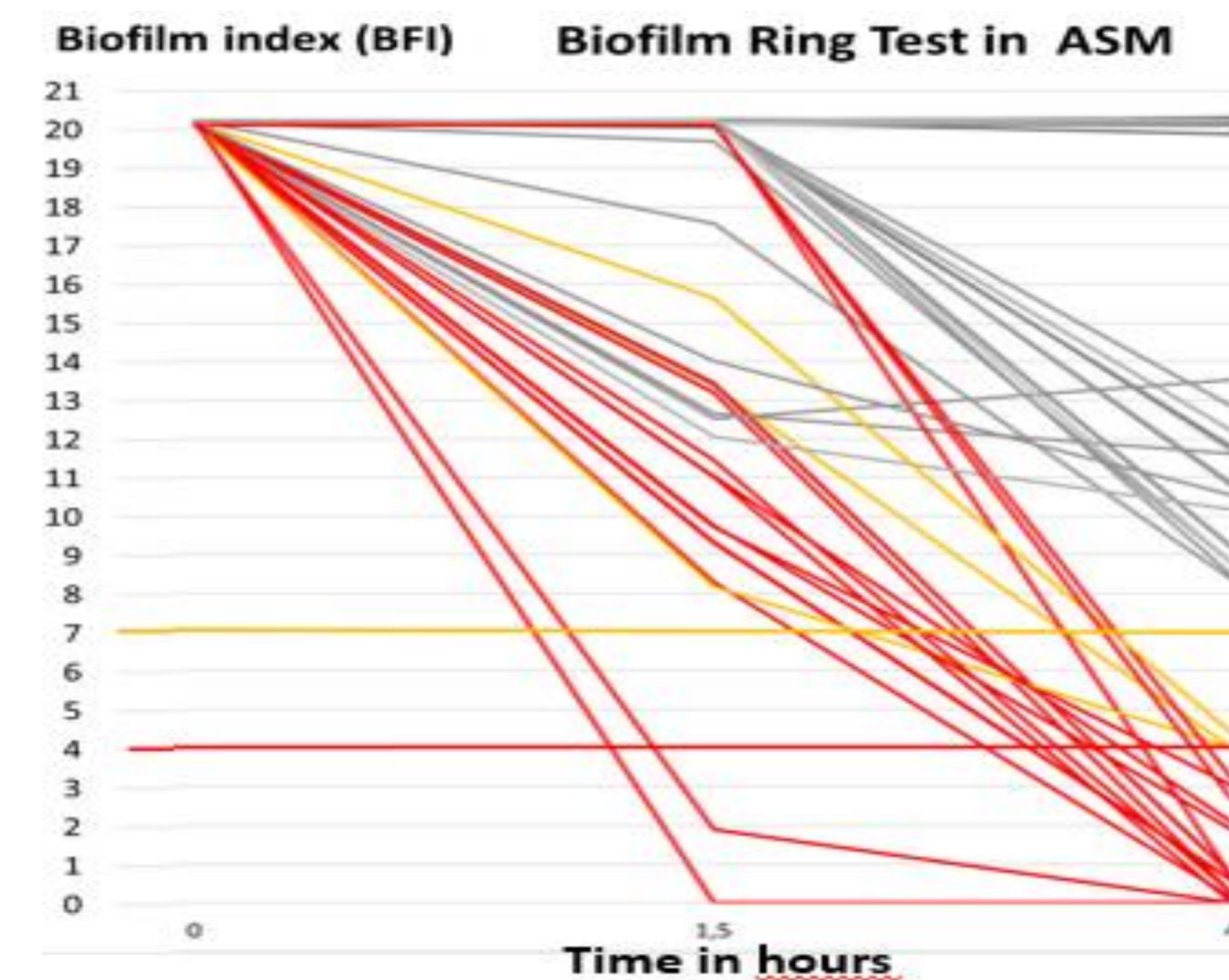


Figure 3: Ability to form biofilm using BRT in ASM. Grey= no biofilm production (BP), orange= partial BP, red= complete BP.

The 17 strains classified as complete biofilm producers were submitted to **Antibiofilmogram®**:

- Antibiotic susceptibility decreased in biofilm** (bMIC > MIC).
- Prevention of biofilm formation (Figure 4) was observed for:**
 - 76.5% of the strains (13/17) with ceftaroline
 - 70.6% of the strains (12/17) with ceftobiprole
 - 60.7% of the strains (11/17) with linezolid
- 5 strains were susceptible to rifampicin and an antibiofilm effect was observed for 3 strains (not shown).
- No antibiofilm activity was noted for trimethoprim, as all the 17 strains (MIC: 8-32 mg/L) exhibited bMICs above the resistance threshold (not shown).

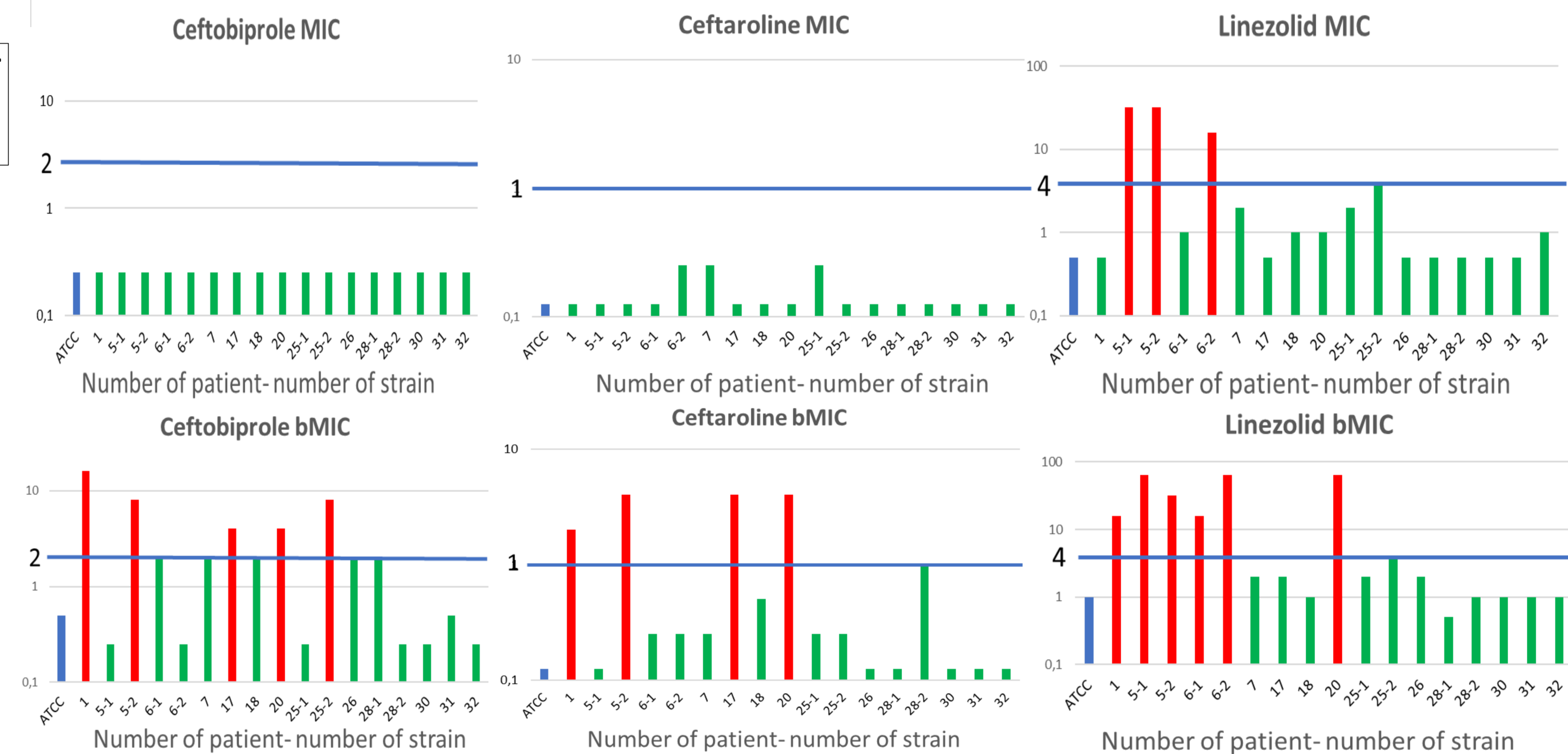


Figure 4: Distribution of MICs (top) and bMICs (bottom) of ceftobiprole, ceftaroline and linezolid. Red= resistant strain, green= susceptible strain. Vertically= MIC or bMIC in mg/L. Horizontally= strain designation (patient number followed by strain number if multiple strains isolated from a sample were tested).

AIM

To evaluate the ability of MRSA isolated from CF patients to form biofilm, in absence and in presence of different antibiotics

METHODS

- 65 MRSA strains isolated from 35 CF patients at the CF center of the University Hospital of Montpellier, France + reference strain ATCC 29213.
- Genotyping by **Multi Locus Sequence Typing (MLST)** → Sequence Type (ST)⁴.
- BioFilm Ring test® (BRT)**⁵ for evaluating:
 - ⇒ the ability to form biofilm in the Artificial Sputum Medium (ASM), mimicking the CF lung environment:
 - * measure of Biofilm index (BFI) after 4h
 - * BFI > 7: weak biofilm production (BP); 4 < BFI < 7: moderate BP; BFI < 4: complete BP
 - ⇒ the Antibiofilmogram⁵ to evaluate the impact of **ceftobiprole, ceftaroline, rifampicin, linezolid and trimethoprim** on the biofilm formation:
 - * determination of the biofilm Minimal Inhibitory Concentrations): **bMICs**
 - * comparison of bMIC with MIC

CONCLUSIONS

- We first report data on early biofilm formation by CF MRSA isolates using an original and standardized approach not previously applied to such isolates⁵.
- Strains of ST5 and ST8 were predominant among the MRSA population studied and more rapidly produced mature biofilm than the other genotypes⁶.
- Antibiotic susceptibility decreased in biofilm highlighting the risk of treatment failure in CF patients.
- Antibiofilmogram® represents a promising tool to guide the choice of the most effective drugs against biofilm formation.
- In our study, prevention of biofilm formation was observed for ceftaroline > ceftobiprole > linezolid.

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CONTACT INFORMATION

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